Myco-4

For the treatment of Mycoplasma-infected cells
Product code A8366

Description

Contamination of cell cultures by mycoplasma is a common problem. Mycoplasma contamination causes biochemical changes as well as changes in the immunological properties of the cells. For both biological and economical reasons, it is important to eliminate mycoplasma from cell cultures being used for basic research, diagnostics, and biotechnological production. The most commonly used method for elimination, inactivation, or suppression of mycoplasma in cell cultures is treatment with antibiotics. But antibiotic therapies may fail to achieve permanent elimination. Also, the cytotoxic properties of antibiotics can cause undesirable side effects on eukaryotic cells and can facilitate the development of resistant mycoplasma strains.

Myco-4 is a combination of a standard antibiotic and a biological reagent. In comparison to most bacteria, mycoplasma lack cell walls but are surrounded by a cytoplasmic membrane. This biological reagent integrates into the mycoplasma membrane and compromises its integrity. By the combination with a standard antibiotic, the effective dose of both, the reagent and the antibiotic, can be reduced to a minimum for lowest cytotoxicity, still causing a highly reliable and definite elimination of mycoplasma. These biophysical properties make the development of resistant strains very unlikely.
One application comprises of 4 vials, a Starter Treatment and three Main Treatment solutions. The Starter Treatment kills most of the mycoplasma particles without harming the cells. The Main Treatment kills all remaining particles leading to a permanent eradication.

A8366,0001: 1 Kit A8366,0002: 2 Kits

Components Each kit contains 1 vial of *Starter Treatment solution* and 3 vials of *Main Treatment solution*. Each component is a sterile, ready-to-use solution, aliquoted per vial for single applications of approx. 520 µl/vial.

Shipment: Ambient temperature

Storage: Store 2-8 °C in the dark. Under these conditions the product is stable until the expiry date given on the bag label.

For research use only

Advantages of Myco-4 over conventionally used antibiotic treatments

- Lowest Cytotoxicity – Cytotoxic effects are very low in most cell lines (Fig.2). Changes in cell morphology are only rarely observed during the treatment.
- Efficiency – Almost 100 % of permanent eradication for mycoplasma is achieved.
- Universal - Myco-4 is suitable for all permanent mammalian cell lines.
- Broad spectrum - Any type of Mycoplasma, Acholeplasma, Spiroplasma, and Entomoplasma can be successfully treated with Myco-4.
- Compatibility - Myco-4 can be used with antibiotic selection agents, for example G418, Blasticidin S, and Hygromycin. It also does not interfere with killer genes turned on by Tetracyclin or Doxycyclin.
- Low resistance risk - Due to the combination of antibiotic and a biophysical mode of action directly killing the mycoplasma, formation of resistances against Myco-4 is most unlikely.

Material needed but not provided with the product:
- Standard cell culture equipment and consumables
- Cell culture medium, fetal calf serum, trypsin
- Mycoplasma detection system to verify the elimination success (e.g. PCR Mycoplasma Test Kit II, Prod. No. A8994)

Protocol

The protocol is designed for typical cell lines requiring standard media. It is suitable for adherent as well as suspension cell lines. **Modification of the protocols may be required for individual cases.**

1. In a sterile culture flasks or petri dish mix 4.5 ml standard cell culture medium (with 5 % v/v FCS) and 500 µl Myco-4 *Starter Treatment* (vial with red cap).
2. Transfer 5 ml of **10^4 - 10^5 single cells** in cell culture medium (with 5% FCS) into the mix. Make sure that the number of cells is correct and that no cell clusters are present!

   The total volume of the treatment mixture is 10 ml, the final serum concentration is 5 % (v/v). Higher serum concentrations will avoid a successful treatment!

   Ensure the treatment of single cells (check under microscope). If necessary increase duration of trypsin treatment or detach the cells from each other by pipetting up and down.

   Ensure that Myco-4 is already present in the culture medium before adding cells. Add cells directly to the elimination mix to avoid evaporation (insert the pipette tip directly into the mix!).

3. Incubate cells until all cells have re-attached (at least 30 min), or, for the common time period of a normal passage.
4. Add 500 µl of the Myco-4 *Main Treatment Reagent* (vial with yellow cap) to 9.5 ml of passaged cells in fresh media. Adjust the FCS concentration. Supplementation to 5 % is not required any longer.
5. Grow the cells to 80-90% confluency, split the cells and passage at the usual rate.
6. Repeat steps 4-5 twice.

   After the third treatment (and a total of 4 passages starting with the *Starter Treatment solution*) the procedure is finished and the culture is free of mycoplasma according to our experience.

Background information, recommendations for optimization

Myco-4 is used for the elimination of Mycoplasma, Acholeplasma, Spiroplasma, and Entomoplasma in all kinds of cell and virus cultures.

For best results the following recommendations should be considered:

1. Not more than 10^5 cells shall be used for a treatment to keep the mycoplasma load low.
2. The mycoplasmacidal activity of Myco-4 is affected by the concentration of lipids and proteins in the reaction mixture, e.g. components in fetal calf serum supplement. These ingredients competitively bind Myco-4 and prevent its binding to the mycoplasma membrane. Our protocol was designed using 5 % v/v of fetal calf serum. Due to the mitigating effect of serum, it is impossible to design a specific protocol that is applicable or the treatment of biologicals with high protein and lipid concentrations.
3. The type of cell culture medium does not affect the efficiency of the treatment. Antibiotics, especially if required for selection, can stay in the elimination mixture. In rare cases cytotoxicity might be increased by unpredictable interactions of the reagents.
4. Viruses shall be treated in combination with their host cells.
5. Only cells harboring Chlamydia or other bacteria as host systems cannot be treated. The antibiotic portion of the product might affect the integrated microorganisms.
6. Myco-4 does not penetrate the cellular membrane. Therefore it cannot eliminate intracellular contamination. However, **mycoplasma is an extracellular contaminant**. *Mycoplasma penetrans* is the only species described to persist intracellular. Thus far, *M. penetrans* has not been reported as contaminant in cell culture.
7. Since Myco-4 works by biophysical means through association with the mycoplasma membrane, the reagent needs direct contact with the mycoplasma particle in order to be effective. Treatment of cell clusters should be avoided. **Mycoplasma are protected in pockets and clefts of the cell membrane, which can prevent contact with the drug. We suggest using trypsin to detach the cells from each other and to smooth cell surfaces.**

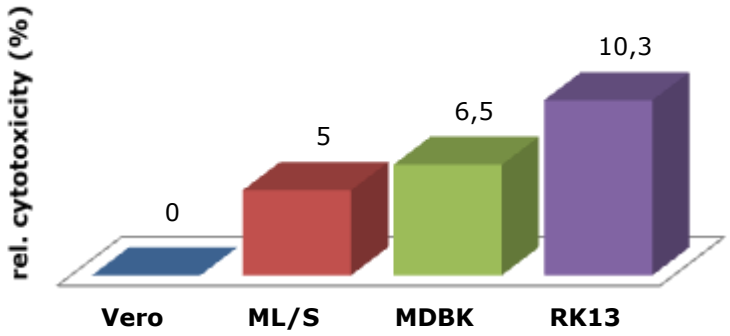

Fig.2 Low Cytotoxicity of Myco-4 on mycoplasma contaminated cells.
Adherent cell lines were treated with Myco-4 according to the protocol 4 days of incubation. Cultures were inactivated and stained as described by Flick and Gifford (1984). Untreated cultures were used as reference (100%).